



HDL cholesterol and apolipoprotein A-I concentrations and risk of atherosclerotic cardiovascular disease

Human genetics to unravel causality

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Published in:
Atherosclerosis

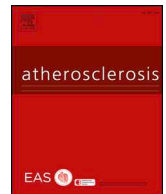
DOI:
[10.1016/j.atherosclerosis.2020.03.005](https://doi.org/10.1016/j.atherosclerosis.2020.03.005)

Publication date:
2020

Document version
Publisher's PDF, also known as Version of record

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Citation for published version (APA):
Frikke-Schmidt, R. (2020). HDL cholesterol and apolipoprotein A-I concentrations and risk of atherosclerotic cardiovascular disease: Human genetics to unravel causality. *Atherosclerosis*, 299, 53-55.
<https://doi.org/10.1016/j.atherosclerosis.2020.03.005>



Apolipoprotein A-I concentrations and risk of coronary artery disease: A Mendelian randomization study

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HIGHLIGHTS

- Apolipoprotein A-I relates to lower observational risk of coronary artery disease.
- Robust evidence of atheroprotective properties of apolipoprotein A-I is lacking.
- Mendelian randomization was used to assess the causality of apolipoprotein A-I.
- Apolipoprotein A-I association with coronary artery disease is likely not causal.
- Apolipoprotein A-I increasing therapies unlikely reduce risk of heart disease.

Abbreviations: apoA-I, apolipoprotein A-I; CAD, coronary artery disease; LD, linkage disequilibrium; MR, Mendelian randomization; NMR, nuclear magnetic resonance; pQTL, protein quantitative trait locus; SNP, single-nucleotide polymorphism

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<https://doi.org/10.1016/j.atherosclerosis.2020.02.002>

Received 12 December 2019; Received in revised form 14 January 2020; Accepted 7 February 2020

Available online 14 February 2020

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ARTICLE INFO

Keywords:

Apolipoprotein
Heart disease
Lipids
Mendelian randomization

ABSTRACT

Background and aims: Apolipoprotein A-I (apoA-I) infusions represent a potential novel therapeutic approach for the prevention of coronary artery disease (CAD). Although circulating apoA-I concentrations inversely associate with risk of CAD, the evidence base of this representing a causal relationship is lacking. The aim was to assess the causal role of apoA-I using human genetics.

Methods: We identified a variant (rs12225230) in *APOA1* locus that associated with circulating apoA-I concentrations ($p < 5 \times 10^{-8}$) in 20,370 Finnish participants, and meta-analyzed our data with a previous GWAS of apoA-I. We obtained genetic estimates of CAD from UK Biobank and CARDIoGRAMplusC4D (totaling 122,733 CAD cases) and conducted a two-sample Mendelian randomization analysis. We compared our genetic findings to observational associations of apoA-I with risk of CAD in 918 incident CAD cases among 11,535 individuals from population-based prospective cohorts.

Results: ApoA-I was associated with a lower risk of CAD in observational analyses (HR 0.81; 95%CI: 0.75, 0.88; per 1-SD higher apoA-I), with the association showing a dose-response relationship. Rs12225230 associated with apoA-I concentrations (per-C allele beta 0.076 SD; SE: 0.013; $p = 1.5 \times 10^{-9}$) but not with confounders. In Mendelian randomization analyses, apoA-I was not related to risk of CAD (OR 1.13; 95%CI: 0.98, 1.30 per 1-SD higher apoA-I), which was different from the observational association. Similar findings were observed using an independent *ABCA1* variant in sensitivity analysis.

Conclusions: Genetic evidence fails to support a cardioprotective role for apoA-I. This is in line with the cumulative evidence showing that HDL-related phenotypes are unlikely to have a protective role in CAD.

1. Introduction

Large-scale cardiovascular outcome trials [1–4] and studies of human genetics [5] do not support a causal role of high-density lipoprotein (HDL) cholesterol in coronary artery disease (CAD). However, it remains feasible that other aspects of HDL, such as the functional attributes of HDL particles, might have atheroprotective effects. The ability of HDL particles to extract cholesterol from lipid-laden cells, so-called cholesterol efflux, has recently emerged as the most prominent new measure for HDL-related atheroprotectivity [6]. Apolipoprotein A-I (apoA-I) is a key functional apolipoprotein component of HDL particles and plays a central role in cholesterol efflux [7]. This has led to anticipation that modification of circulating apoA-I might represent a novel therapeutic approach to the treatment and prevention of CAD. Notably, Pfizer reportedly paid over 1 bn USD for the commercial rights for an apoA-I infusion technology [8].

Although epidemiological studies have shown that circulating concentrations of apoA-I are strongly inversely associated with CAD [9], recent phase II randomized controlled trials (RCTs) that used MDCO-216 (recombinant apoA-I Milano) and CER-001 (recombinant wild-type apoA-I) infusions failed to identify a beneficial effect of apoA-I infusion on the regression of coronary atherosclerosis as measured by intravascular ultrasonography [10,11], resulting in the termination of the development of these apoA-I products. Regardless of these failures, a third apoA-I infusion therapy, CSL112, representing a reconstituted form of native apoA-I from human plasma, is currently underway in a large phase III RCT (AEGIS-II; [ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT03473223) Identifier: NCT03473223), which seeks to assess the efficacy of CSL112 on the risk of cardiovascular events [12,13]. Prior trials have shown CSL112 to be well-tolerated [14,15], and capable of increasing HDL cholesterol efflux in both healthy individuals and CAD patients [16–18].

Despite the ongoing apoA-I trial, robust evidence of atheroprotective properties of apoA-I is lacking. Human genetics can yield reliable evidence on the likely efficacy of pharmacological modification of a therapeutic target on risk of disease. For example, genetic variants in *HMGCR* and *PCSK9* reliably elucidate the effects of statins and PCSK9 inhibition on circulating metabolic markers (including lipoproteins and their accompanying lipids) and risk of CAD [19,20]. In a recent study, Richardson et al. [21] used the UK Biobank data to construct multi-marker genetic instruments for plasma lipid traits (including apoA-I and HDL cholesterol) to assess their causal roles in CAD using univariable and multivariable Mendelian randomization (MR) analyses. Although

the univariable MR approach suggested that apoA-I and HDL cholesterol might be protective of CAD, the findings diminished to the null in multivariable analysis taking apolipoprotein B (apoB) into account. These findings call attention on the overall validity of genetic instruments in MR analyses for highly correlated lipoprotein-related measures, such as apoA-I. There is a high likelihood for horizontal pleiotropy through various biological pathways and mechanisms when multiple variants are selected from across the genome. In the current study, we used human genetics in five population-based cohorts with detailed lipoprotein profiling together with large-scale genome-wide data to identify a robust genetic instrument for apoA-I concentrations in order to provide reliable estimates of the potential causal effects of apoA-I on risk of CAD. In an effort to overcome the potential issue of horizontal pleiotropy, we aimed to construct an instrument that would be as free as possible from confounding and therefore our primary focus was on the locus containing the gene encoding apoA-I. We also compared our genetic results to observational associations of apoA-I concentrations and the risk of incident CAD in population-based cohorts.

2. Materials and methods

A diagram showing our primary approach to assess causality of circulating apoA-I concentrations in CAD is shown in [Supplemental Fig. S1](#). We used individual participant data including genotyping and lipoprotein profiling from five Finnish population-based cohorts, totaling up to 20,370 individuals. Please see Supplementary Material for detailed descriptions of the cohorts ([Supplemental Text S1, S2 and S3](#); [Supplemental Tables S1 and S2](#)), details of analyses ([Supplemental Text S4 and S5](#)), and assessment of observational estimates for CAD ([Supplemental Text S6](#)).

2.1. Identifying genetic instruments for the circulating apoA-I concentration

Our aim was to develop a genetic instrument that would mimic the effect of apoA-I infusion therapies; i.e. we aimed to find genetic variants that would be associated with increased circulating apoA-I concentrations without being confounded by associations with lipid traits and phenotypes, other than those that would be expected due to vertical pleiotropy. To this end, we focused on *cis*-acting protein quantitative trait loci (pQTL) in the *APOA1* locus, which contains the gene encoding apoA-I. We used this approach, because apoA-I associated variants from the apoA-I encoding locus represent the instruments plausibly least

likely to violate the exclusion restriction criterion, being plausibly linked to apoA-I concentrations through *cis*-effects. We also investigated whether construction of a multiallelic instrument including all *cis* and *trans* pQTLs from loci associated with apoA-I would be feasible in our cohorts. We investigated the effects of previously reported apoA-I associated SNPs across a wide range of lipoprotein lipids and metabolites (Supplemental Table S3) and detected that construction of such an instrument was not possible due to pleiotropy across multiple lipoprotein measures including apoB (i.e., most apoA-I associated SNPs had confounding associations with several lipoprotein lipids; see Supplemental Text S7 and Supplemental Figs. S2, S3, and S4 for details). However, one of the apoA-I associated variants (rs2740486 in *ABCA1* locus) was not associated with apoB-related cholesterol measures and was used in sensitivity analysis to validate the associations of the *cis*-pQTL instrument (see Supplemental Text S7 and S8). We primarily concentrated on the 1-Mb region flanking the *APOA1* gene to find genetic variants associated with circulating apoA-I concentrations. Our initial approach was to count the number of single-nucleotide polymorphisms (SNPs) in this region and use this value to correct the multiple testing through a conventional Bonferroni approach, which corresponded to 7,210 SNPs and a Bonferroni-adjusted *p*-value of $< 7 \times 10^{-6}$. However, the top SNPs superseded this threshold and associated with apoA-I concentrations at conventional levels of GWAS significance ($p < 5 \times 10^{-8}$). We compared our results to those of a previous GWAS of apoA-I [22] and prioritized variant rs12225230 as the genetic instrument for apoA-I; this SNP was reported to be associated with apoA-I concentrations in the previous GWAS [22] and summary statistics were available from this study. To identify whether more than one *cis*-pQTL associated with apoA-I, we repeated the analysis of apoA-I concentrations on SNPs in/around *APOA1* after conditioning on rs12225230. No additional conditionally-independent *cis*-pQTLs were identified. Details of the analyses, selection criteria, and consideration of validity of our instrument are described in Supplementary Material (Supplemental Text S7, Supplemental Table S4).

2.2. Assessing causality

We assessed causality between the exposure (circulating apoA-I concentrations) and outcome (CAD) utilizing the Mendelian randomization (MR) framework (Supplemental Fig. S1). We used a two-sample MR approach where summary data of the exposure (i.e., SNP to apoA-I concentration) and outcome (i.e., SNP to CAD) originate from different sources [23,24]. Rs12225230 located in the *APOA1* locus was used as the genetic instrument. This approach included the use of the following estimates [1]: per-allele effect estimate (in g/L) for the association between rs12225230 and apoA-I (meta-analyzed result between the Finnish individual participant data and a prior GWAS [22]; total $N = 37,093$) [2]; per-allele effect estimates with risk of CAD from a large GWAS, i.e., meta-analysis of the UK Biobank with CARDIoGRAMplusC4D [25] (case $N = 122,733$). To obtain a causal estimate and the corresponding standard error for the association between apoA-I and CAD, we used the inverse-variance weighted method [26] that collapses to Wald ratio for a single variant. To facilitate comparison with observational estimates, the causal estimate of apoA-I concentrations on risk of CAD was scaled to SD units, using SD of 0.225 g/L (the SD of apoA-I in our largest cohort, FINRISK97). We assessed evidence of heterogeneity between the causal and observational estimates using Cochran's Q statistic. In sensitivity analyses, we repeated the MR analysis using the per-allele effect estimate between rs12225230 and apoA-I using values derived from just the Finnish population cohorts and just the prior GWAS [22]. We also repeated the MR analysis using another unconfounded apoA-I associated SNP (rs2740486 in *ABCA1* locus) as an instrument as a sensitivity analysis (see Supplemental Text S7 and S8). Similar to analysis with rs12225230 as the instrument, we used the meta-analyzed result of rs2740486 between the Finnish individual participant data and a prior GWAS [22] in this analysis.

Finally, we created an instrument using both rs12225230 and rs2740486 and repeated the MR analysis using the inverse-variance weighted method [26]. We report our power to detect various effect estimates of apoA-I concentrations and risk of CAD in the MR analyses in Supplemental Table S4.

3. Results

3.1. Identification of genetic variants associated with circulating apoA-I concentrations

Multiple SNPs in/around the *APOA1* locus were associated with apoA-I serum concentration (Fig. 1, Supplemental Table S5) with the association peak spanning a 466-kb region. We identified rs12225230 to associate with apoA-I concentrations at GWAS significance (per-allele beta 0.076 SD, $p = 1.5 \times 10^{-9}$), which was in linkage disequilibrium (LD) with the top SNP rs625145 ($D' = 1.00$, $r^2 = 0.91$). This variant was previously shown to be associated with apoA-I serum concentration [22] and is mapped to the SIK family kinase gene (*SIK3*) encoding serine/threonine protein kinase SIK3, located 20 kb upstream of *APOA1*. When we conditioned on rs12225230, there were no additional associations (Supplemental Fig. S5), indicating that rs12225230 or SNPs in LD with this SNP are likely the principal drivers of the apoA-I association in this locus. We estimated the effect of the minor allele of rs12225230 on apoA-I concentrations to be 0.0158 g/L (SE 0.0030). In meta-analysis of our cohorts with a previous GWAS of apoA-I concentrations (reporting 0.0320 g/L [SE 0.0033] increment per minor allele of rs12225230) [22] the effect of the minor allele C of rs12225230 was estimated to be 0.0230 g/L (SE 0.0022; $p = 1.5 \times 10^{-25}$), corresponding to an F-statistic of 109. We further investigated whether any SNPs located in the *APOA1* gene were associated with apoA-I, and found that a single SNP, rs670 located in the 5' untranslated region of *APOA1* that was also previously shown to be associated with apoA-I concentrations [27], associated with apoA-I concentrations (per-allele beta 0.070 SD, $p = 3.8 \times 10^{-8}$; Supplemental Table S5); however, this SNP is in LD ($D' = 0.90$, $r^2 = 0.81$) with rs12225230 and thus likely represents the same association signal.

We assessed whether rs12225230 represents an unbiased instrument to mimic the effect of apoA-I increasing therapies by characterizing the effects of rs12225230 on metabolic measures (Fig. 2, Supplemental Fig. S3). We detected that, in addition to associating with higher apoA-I concentrations, the minor allele of rs12225230 was associated with higher levels of multiple HDL-related measures, including, e.g., concentration of large, medium and small HDL particles (L-HDL-P, beta 0.055 SD, $p = 1.2\text{E-}05$; M-HDL-P, beta 0.061 SD, $p = 1.2\text{E-}06$; S-HDL-P, beta 0.055 SD, $p = 1.2\text{E-}05$), and HDL cholesterol (beta 0.070 SD, $p = 2.5\text{E-}08$). In addition, rs12225230 was associated, e.g., with fatty acid measurements (e.g., polyunsaturated fatty acids, beta 0.048 SD, $p = 1.2\text{E-}04$); associations with the fatty acids likely reflect their associations with multiple HDL-related traits. In contrast, rs12225230 was not associated with the majority of apoB related traits (Supplemental Fig. S3). Rs12225230 showed the strongest associations with apoA-I; associations with the other traits were weak or null. Because our aim was to develop an instrument mimicking apoA-I infusion therapy (and treatment with such is recognized to cause an increase in both apoA-I and HDL cholesterol concentrations [15]), associations of our genetic instrument with HDL-C related traits should not represent confounding in this setting. Thus, rs12225230 likely represents an unbiased instrument for apoA-I concentrations. We estimated that the proportion of variance in apoA-I concentrations explained by rs12225230 was 0.32%. We further estimated that we had adequate ($> 90\%$) power to detect an effect estimate of OR 0.80 for CAD using rs12225230 as the genetic instrument (Supplemental Table S4). Further considerations of validity of the instrument are given in Supplemental Text S7.

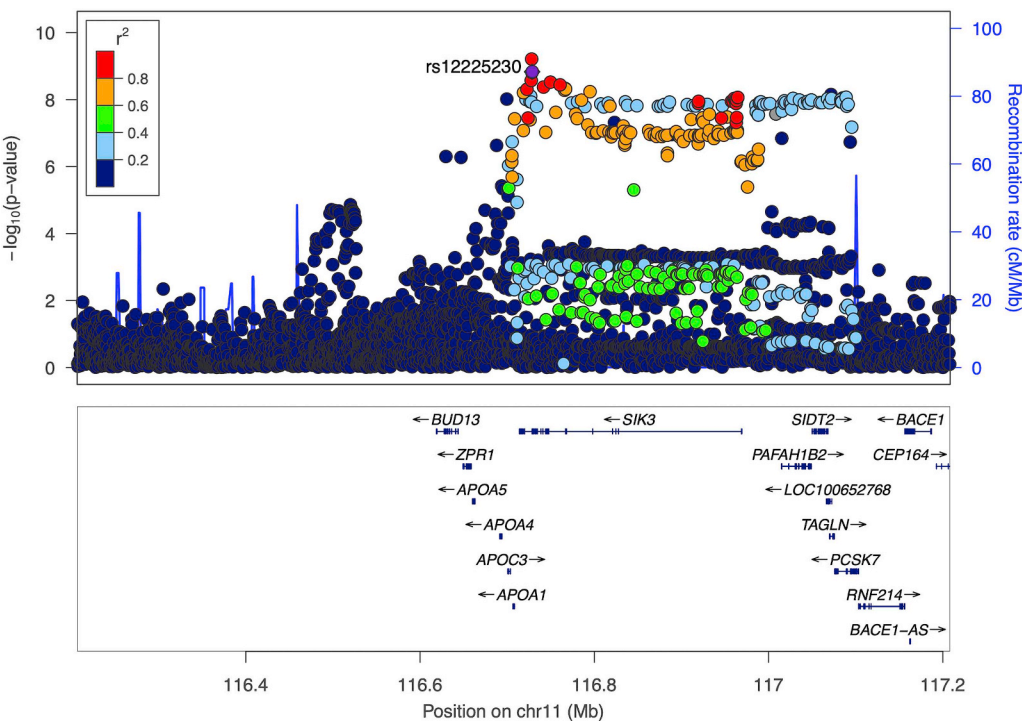


Fig. 1. Association of SNPs in the *APOA1* locus with circulating concentrations of apoA-I. Each dot represents the association of a single genetic variant with apoA-I concentrations in meta-analysis of five Finnish population cohorts ($-\log_{10}$ of p value shown on the y axis and chromosomal position on the x axis). The 500-kb region flanking *APOA1* is shown. Rs12225230 (highlighted in violet; used as an instrument in this study) is robustly associated with apoA-I concentrations ($p = 2 \times 10^{-3}$) and is in LD with the top variant rs625145 ($p = 6 \times 10^{-10}$). Colors of the variants refer to their r^2 values with rs12225230 in the European population.

We further investigated whether the association of rs12225230 with apoA-I concentration could be confounded by associations with other phenotypes. To this end, we screened publicly available genotype-phenotype associations (altogether 2,991 diseases and traits in a phenome-wide association analysis) using PhenoScanner [28]. The associations of rs12225230 were not likely confounded by associations with other phenotypes, including conventional risk factors (type 2 diabetes, systolic blood pressure, BMI, smoking, or alcohol consumption; see Supplemental Text S7, Supplemental Table S6, Supplemental Fig. S6). In sensitivity analysis, we used an unconfounded SNP (rs2740486) from another apoA-I associated locus, *ABCA1*, as the instrument. Similar to rs12225230, we verified that this SNP was unlikely to be confounded through associations with other traits (see Supplemental Text S7 and S8, Supplemental Fig. S4).

3.2. Observational associations of apoA-I concentrations with incident coronary artery disease

We estimated the prospective observational associations of apoA-I concentrations with risk of CAD in the FINRISK97 cohort ($N = 7,133$, with 743 incident CAD events), and compared the association of apoA-I

to the corresponding CAD associations for apoB, HDL and LDL cholesterol. In the comparisons of the lipid measures, the associations were assessed using two models: a minimal model (age and sex as covariates) and a fully adjusted model in which conventional risk factors for CAD (age, sex, body mass index, systolic blood pressure, type 2 diabetes, smoking and alcohol consumption) were included as covariates. ApoA-I concentrations (HR 0.86 per 1-SD higher apoA-I; 95%CI 0.79–0.93, Fig. 3) had a similar magnitude of association with CAD as did HDL cholesterol (HR 0.79 per 1-SD higher HDL-C; 95%CI 0.72–0.86), apoB (HR 0.84 per 1-SD lower apoB; 95%CI 0.79–0.91) and LDL cholesterol (HR 0.88 per 1-SD lower LDL-C; 95%CI 0.82–0.95). The associations were similar in minimally and fully adjusted models (Supplemental Table S7). The association of apoA-I concentrations with CAD showed a dose-response relationship, with the risk of CAD being HR 0.57 (95%CI 0.45–0.75) comparing the highest to lowest quintiles of apoA-I (Fig. 3B), adjusted for the covariates in the fully adjusted model above together with LDL cholesterol.

In meta-analysis of the FINRISK97 and FINRISK07 cohorts (total 918 incident CAD cases), higher concentrations of apoA-I associated with a lower risk of CAD (HR 0.81 per 1-SD higher apoA-I, 95%CI 0.75–0.88) after adjustment for age, sex, and conventional risk factors

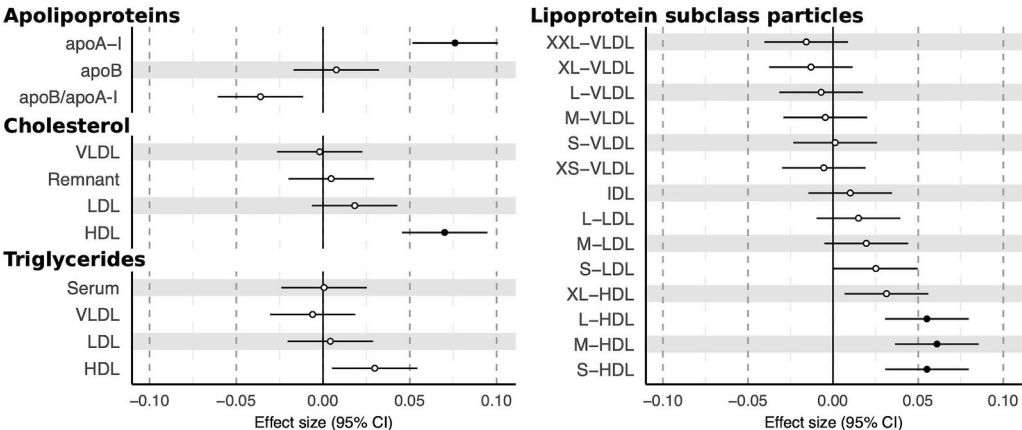


Fig. 2. Association of rs12225230 with key lipoprotein-related concentration measures. Remnant cholesterol refers to cholesterol carried in VLDL and IDL particles. Effect sizes are estimated as SD differences in metabolite concentrations per rs12225230-C allele. Closed symbols, $p < 0.002$ (significant association); open symbols $P \geq 0.002$. Abbreviations: apoA-I, apolipoprotein A-I; apoB, apolipoprotein B; VLDL, very low-density lipoprotein; LDL, low-density lipoprotein; HDL, high-density lipoprotein; IDL, intermediate-density lipoprotein.

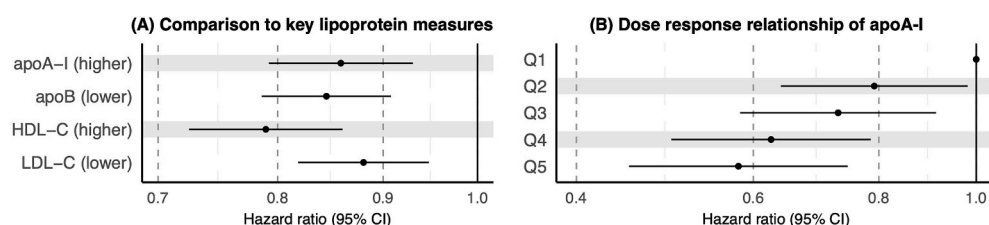


Fig. 3. Observational associations of apoA-I concentrations and risk of coronary artery disease.

(A) The association of apoA-I concentrations with CAD risk was compared to those of key lipoprotein measures (apoB, HDL and LDL cholesterol). Note, the effects of apoB and LDL cholesterol are inverted; i.e., the effects are shown per 1-SD reduction in

apoB and LDL cholesterol. Associations were assessed with 743 cases and 6,390 controls in FINRISK97, with adjustment for age, sex, body-mass index, systolic blood pressure, type 2 diabetes, smoking and alcohol consumption. Associations are presented as HR (95%CI) per 1-SD lipoprotein measure. (B) Dose-response association of apoA-I concentrations with risk of CAD. Associations were additionally adjusted for LDL cholesterol. Effect estimates are presented per quintile of apoA-I with the lowest quintile (Q1) used as the reference category.

for CAD (LDL cholesterol, body-mass index, systolic blood pressure, type 2 diabetes, smoking and alcohol consumption). The associations were similar in minimally (age and sex as covariates) and fully (age, sex, LDL cholesterol, body-mass index, systolic blood pressure, type 2 diabetes, smoking and alcohol consumption as covariates) adjusted models and in both cohorts (Supplemental Fig. S7). ApoA-I concentrations showed only low correlation (r between -0.3 and 0.3) with the potential confounders included as covariates in the models (Supplemental Fig. S8).

3.3. Evaluation of the causal role of serum apoA-I concentrations in coronary artery disease

Using data from a large GWAS of coronary artery disease including 122,733 CAD cases in 547,261 individuals (meta-analysis of UK Biobank CAD GWAS with CARDIoGRAMplusC4D [25]), rs12225230 was not associated with risk of CAD (per-allele log-odds 0.0124, SE 0.0073, $p = 0.090$). Similarly, no significant association of rs12225230 was reported in any of the large GWAS for cardiovascular phenotypes, including CAD, MI or stroke (Supplemental Table S8). Rs670, located in 5' untranslated region of *APOA1*, was not associated with CAD either (per T-allele log-odds 0.0171, SE 0.0077, $p = 0.025$).

Using the rs12225230-apoA-I and rs12225230-CAD associations, we did not find evidence to support a causal role of apoA-I concentrations with risk of CAD: the causal estimate (calculated based on meta-analysis of our own GWAS and a previous GWAS of apoA-I [22]) for CAD was an OR of 1.13 per 1-SD higher apoA-I concentration, 95%CI 0.98–1.30 (Fig. 4, Supplemental Fig. S9) which differed (p -heterogeneity < 0.001) to the corresponding fully-adjusted (age, sex, LDL cholesterol, body-mass index, systolic blood pressure, type 2 diabetes, smoking and alcohol consumption as covariates) observational estimate (HR 0.81; 95%CI: 0.75–0.88). Causal estimates for CAD were similar

when calculated based on the apoA-I effect estimates from the previous GWAS [22] (OR 1.09, 95%CI 0.99–1.21) and our own GWAS (OR 1.19, 95%CI 0.97–1.46). Similarly, there was no evidence of apoA-I concentrations having a causal role in MI (OR 1.19, 95%CI 0.93–1.53) or stroke (OR 0.87, 95%CI 0.70–1.07).

All of the above analyses were also conducted using an unconfounded apoA-I associated variant from another locus (rs2740486 in *ABCA1*). Similarly to rs12225230, this SNP was not associated with CAD (per T-allele log-odds 0.0049, SE 0.0051, $p = 0.343$) in meta-analysis of UK Biobank CAD GWAS with CARDIoGRAMplusC4D [25], and there was no evidence supporting a causal role of apoA-I concentrations with risk of CAD (causal estimate being an OR of 1.08 per 1-SD higher apoA-I concentration, 95%CI 0.92–1.27, p -heterogeneity = 0.002, Supplemental Fig. S10). Finally, we repeated the MR analysis utilizing an instrument consisting of *APOA1*-rs12225230 and *ABCA1*-rs2740486: the causal estimate for CAD was an OR 1.11 (95%CI 1.00–1.23, $p = 0.056$, p -heterogeneity < 0.001 , Supplemental Fig. S10).

4. Discussion

Our findings do not provide genetic support for the hypothesis that circulating apoA-I concentrations are protective of CAD. Together with previous evidence indicating that HDL cholesterol does not have a protective effect on risk of CAD [1,2,5], these results suggest that apoA-I may not represent a valid therapeutic approach for the prevention and treatment of CAD.

HDL cholesterol and apoA-I are among the serum lipoprotein measures showing strongest inverse associations with cardiovascular disease in epidemiological studies [9]. However, RCTs using HDL cholesterol increasing therapies have failed to identify that the risk of CAD is proportionate to the amount by which HDL cholesterol is increased [1,2] and, consistently, studies of human genetics find that the association of HDL cholesterol with CAD is not causal [5]. In the REVEAL trial of the cholesteryl ester transfer protein (CETP) inhibitor, anacetrapib, although treatment with anacetrapib did increase HDL cholesterol, the cardiovascular benefit was proportionate to the degree of apoB lowering, rather than to the effect on HDL cholesterol [3]. Here, we add further evidence showing that targeting HDL-related pathways through increases in apoA-I may not effectively lower risk of CAD. We show that long-term genetically increased circulating apoA-I concentrations do not protect from CAD, a finding consistent with the outcomes in two trials of apoA-I infusion products (MDCO-216 and CER-001) that led to the termination of development of those products [10,11]. Thus, randomized evidence of both the short-term, transient increase in circulating concentration of apoA-I arising from apoA-I infusions and the life-long increase caused by genetic variants fails to support a protective role of apoA-I concentrations in CAD.

This study has several strengths. The SNP used to mimic the effect of apoA-I-increasing therapy, rs12225230, was identified in a locus-specific analysis of SNPs in/around *APOA1* in five Finnish cohorts, replicating the finding of a prior GWAS [22]. By focusing on *cis*-acting pQTL SNPs from the *APOA1* locus, we sought to minimize the potential

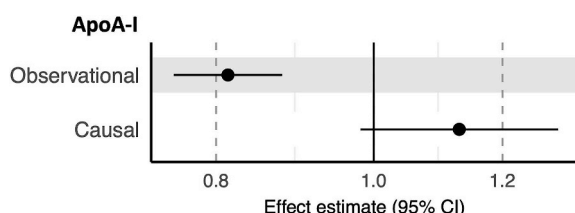


Fig. 4. Observational and causal effects of circulating apoA-I concentrations and risk of coronary artery disease.

Observational estimate was assessed in the FINRISK97 and FINRISK07 cohorts (918 coronary artery disease cases among 15,353 individuals) with age, sex, LDL cholesterol, body-mass index, systolic blood pressure, type 2 diabetes, smoking and alcohol consumption as covariates. Causal estimate was calculated based on association of rs12225230 with apoA-I concentrations (effect estimate from meta-analysis of five Finnish cohorts and a previous GWAS [22], total $N = 37,0930$) and coronary artery disease (effect estimate from meta-analysis of the UK Biobank and CARDIoGRAMplusC4D; 122,733 CAD cases among 547,261 individuals). Effect estimates correspond to the risk (observational association: hazard ratio, HR; causal estimate: odds ratio, OR) of CAD per 1-SD higher apoA-I (equivalent to 0.225 g/L apoA-I).

for confounding by *trans*-acting variants [24]. We recognize, however, that the SNP, while being located within 20 kb of the *APOA1* gene and therefore qualifying as a 'cis-pQTL', is nonetheless annotated as a missense variant in a gene (*SIK3*) discrete to that which encodes apoA-I (*APOA1*). There therefore remains the possibility of confounding through functional attributes of the rs12225230 variant mediated through *SIK3*. However, *SIK3* has been shown to be involved in skeletogenesis [29], and therefore it is unlikely that a skeletal developmental gene would confound the associations. To investigate this in greater detail, we assessed the effects of rs12225230 on a detailed panel of serum metabolic measures as a means of providing evidence against potential confounding by circulating metabolic markers other than those related to HDL pathways, and supplemented this by examining potential phenotype associations. We also repeated the MR analysis using an apoA-I associated variant from another locus (*ABCA1*) and the results were consistent. *ABCA1* encodes ATP-binding cassette sub-family member 1, a protein involved in the transport of various molecules across membranes; of note, *ABCA1* actively mediates cholesterol efflux and therefore has particular relevance to our question of whether apoA-I, acting to promote cholesterol efflux, leads to a reduction in risk of CHD [30]. The stronger two-SNP instrument showed clear deviation from the observational estimate. For all analyses, we utilized large, well-characterized Finnish population cohorts and the largest available international GWAS consortia data for CAD to maximize statistical power. Taken together, our statistical and genetic approach yielded reliable estimates of the causal effect of apoA-I on risk of CAD and facilitated the discovery that, unlike the observational association, which is vulnerable to confounding and bias, apoA-I is unlikely to play a causal role in CAD.

Importantly, our results indicating no causal role for apoA-I concentrations in CAD are in line with the results of a recent large multivariable MR study performed using the UK BioBank data. In this study, Richardson et al. [21] first performed univariable MR analysis using a multi-SNP instrument consisting of > 400 independent apoA-I associated SNPs, which indicated a potential causal role for apoA-I in lowering the risk of CAD. However, the authors identified that a large proportion of the SNPs included in the apoA-I instrument were associated with other lipid traits and were thus likely widely affected by horizontal pleiotropy. As an example, their instrument for apoA-I concentrations showed inverse associations with apoB and triglycerides. To overcome this problem, they performed multivariable MR taking into account the effects of apoB; these analyses resulted in a conclusion that apoB, but not apoA-I (or HDL cholesterol), is causally associated with CAD. Our approach, in which we used a *cis*-instrument for apoA-I (a SNP from the apoA-I encoding locus that was not associated with apoB-related lipid traits), is in agreement with the multivariable MR result for apoA-I.

The main focus of investigation was to provide genetic evidence on the hypothesis of whether apoA-I infusions protect from CAD, and to do so, we developed a genetic instrument that strongly and specifically associates with circulating apoA-I concentrations. However, the rationale behind apoA-I-infusion therapies is that increases in apoA-I ought to promote cholesterol efflux from arterial wall macrophages, a process that can be quantitatively measured by HDL cholesterol efflux capacity (HDL-CEC). Similar to HDL cholesterol and apoA-I, HDL-CEC is inversely associated with risk of CAD [6]. The AEGIS trial showed that apoA-I infusion therapy with CSL112 led to higher concentrations of apoA-I and higher HDL-CEC [15,18], which suggests that higher HDL-CEC may represent a target-mediated effect of elevations in apoA-I. If this were true, then we would expect that our genetic instrument for higher apoA-I concentrations would also lead to higher HDL-CEC. Therefore, we would expect that any effect of apoA-I infusion therapy on HDL-CEC ought to be represented by our genetic instrument. Of note, the neutral association in Mendelian randomization of the apoA-I associated *ABCA1* variant with CAD provides important evidence which potentially speaks to a non-causal role of HDL-CEC. Furthermore, in

addition to our findings, studies have indicated that either rare or common variants in the *APOA1* (associated with apoA-I concentrations) and *ABCA1* (associated with HDL cholesterol) genes are not associated with cardiovascular disease [27,31].

Taken together, multiple lines of evidence (including our MR results for apoA-I) clearly indicate that HDL cholesterol and apoA-I are likely not to have atheroprotective effects [1–5,21,32]. Future studies will show if the same is also true for HDL-CEC; indeed, some studies have suggested that cholesterol efflux capacity may associate with cardiovascular disease independent of HDL cholesterol and apoA-I [33]. In addition, it should be noted that despite the findings in human studies, several animal studies have suggested that apoA-I could be a causal atheroprotective factor [34–36]. However, although the first apoA-I trial indicated that apoA-I Milano/phospholipid complex infusions may regress coronary atherosclerosis [37], subsequent RCTs including the recently terminated MDCO-216 and CER-001 trials [10,11] have failed to find a beneficial effect of apoA-I infusions, which, together with our results, strongly indicate a non-causal role for apoA-I in humans.

A common criticism of drug-target Mendelian randomization analyses is that the genetic association with the biomarker of interest (in this case apoA-I) is typically small, and thus meaningful deductions cannot be made about the likely impact of modifying apoA-I through a therapeutic (such as an infusion), where the effect of the intervention on the biomarker of interest is typically much larger. The counter-argument is that atherosclerosis is a life-long disease, and exposure to a small but consistent genetic elevation in apoA-I over several decades ought to lead to a lower risk of vascular disease if apoA-I is truly cardioprotective. It is this 'cumulative' exposure in the setting of a disease with a long latency period (such as cardiovascular disease) which means that 'physiological' risk factors such as LDL cholesterol tend to have much larger effects on risk of cardiovascular disease from Mendelian randomization than from conventional observational studies [24]. In our study, we had good power (> 90%) to detect an effect estimate from Mendelian randomization which was similar to that which we identified from our observational analyses. A false negative might arise were there to be a physiological threshold above which apoA-I is cardioprotective but below which it has no effect. To investigate the plausibility of such a threshold effect, our observational analyses, in which we generated quintiles based on measured values of apoA-I concentrations, showed a clear dose-response relationship between apoA-I concentrations and risk of vascular disease, which argues against such a threshold effect.

This study provides an example of using human genetics to guide the development of therapeutic targets and predict the effects of phase III cardiovascular outcome trials [38]. We note the similarity to other 'negative' MR studies of secretory phospholipase IIA-A₂ [39] and Lp-PLA₂ [40], both of which were the focus of large-scale phase III cardiovascular outcome trials. The cumulative evidence, including genetic evidence from the recent multivariable MR study of lipid traits [21] and our study, brings into question whether pharmaceutical research and development should continue to invest in developing apoA-I infusion therapies for preventing CAD.

In conclusion, our findings do not support the hypothesis that increasing circulating apoA-I concentrations represents a valid approach for the prevention of CAD. These results add to the burgeoning evidence that refutes a protective role of HDL and HDL-related phenotypes in the etiology of CAD.

Financial support

MAK and JK are supported by a research grant from the Sigrid Juselius Foundation, Finland. JK is supported through funds from the Academy of Finland (grant numbers 297338 and 307247) and the Novo Nordisk Foundation (grant number NNF17OC0026062). MVH works in a unit that receives funding from the UK Medical Research Council and is supported by a British Heart Foundation Intermediate Clinical

Research Fellowship (FS/18/23/33512) and the National Institute for Health Research Oxford Biomedical Research Centre. QW is supported by a Postdoctoral Fellowship from the Novo Nordisk Foundation (NNF17OC0027034). VS is supported by the Finnish Foundation for Cardiovascular Research. KK is supported by the Academy of Finland (grant number 250207). The Young Finns Study has been financially supported by the Academy of Finland: grants 286284, 134309 (Eye), 126925, 121584, 124282, 129378 (Salve), 117787 (Gendi), and 41071 (Skidi); the Social Insurance Institution of Finland; Competitive State Research Financing of the Expert Responsibility area of Kuopio, Tampere and Turku University Hospitals (grant X51001); Juho Vainio Foundation; Paavo Nurmi Foundation; Finnish Foundation for Cardiovascular Research; Finnish Cultural Foundation; The Sigrid Juselius Foundation; Tampere Tuberculosis Foundation; Emil Aaltonen Foundation; Yrjö Jahnsson Foundation; Signe and Ane Gyllenberg Foundation; Diabetes Research Foundation of Finnish Diabetes Association; EU Horizon 2020 (grant 755320 for TAXINOMISIS); European Research Council (grant 742927 for MULTIEPIGEN project); and Tampere University Hospital Supporting Foundation. NFBC66 received financial support from University of Oulu Grant no. 65354; Oulu University Hospital Grant no. 2/97, 8/97; Ministry of Health and Social Affairs Grant no. 23/251/97, 160/97, 190/97; National Institute for Health and Welfare, Helsinki Grant no. 54121; and Regional Institute of Occupational Health, Oulu, Finland Grant no. 50621, 54231. NFBC86 was supported by the following grants: EU QLGI-CT-2000-01643 (EUROBLCS) Grant no. E51560; NorFA Grants no. 731, 20056, 30167; and USA/NIH 2000 G DF682 Grant no. 50945. The Baker Institute is supported in part by the Victorian Government's Operational Infrastructure Support Program. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

CRediT authorship contribution statement

Minna K. Karjalainen: Conceptualization, Methodology, Formal analysis, Investigation, Validation, Writing - original draft, Writing - review & editing. **Michael V. Holmes:** Conceptualization, Methodology, Formal analysis, Investigation, Validation, Writing - original draft, Writing - review & editing, Supervision, Project administration, Funding acquisition. **Qin Wang:** Conceptualization, Methodology, Writing - original draft, Writing - review & editing. **Olga Anufrieva:** Formal analysis, Investigation, Validation, Writing - review & editing. **Mika Kähönen:** Resources, Data curation, Writing - review & editing. **Terho Lehtimäki:** Resources, Data curation, Writing - review & editing. **Aki S. Havulinna:** Resources, Data curation, Writing - review & editing. **Kati Kristiansson:** Resources, Data curation, Writing - review & editing. **Veikko Salomaa:** Resources, Data curation, Writing - review & editing. **Markus Perola:** Resources, Data curation, Writing - review & editing. **Jorma S. Viikari:** Resources, Data curation, Writing - review & editing. **Olli T. Raitakari:** Resources, Data curation, Writing - review & editing. **Marjo-Riitta Järvelin:** Resources, Data curation, Writing - review & editing. **Mika Ala-Korpela:** Conceptualization, Methodology, Resources, Data curation, Writing - original draft, Writing - review & editing, Supervision, Project administration, Funding acquisition. **Johannes Kettunen:** Conceptualization, Methodology, Formal analysis, Investigation, Validation, Resources, Data curation, Writing - original draft, Writing - review & editing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

VS has participated in a conference trip sponsored by Novo Nordisk and received an honorarium for participating in an advisory board meeting (unrelated to the present study). He also has ongoing research collaboration with Bayer Ltd (unrelated to the present study). No other authors reported conflicts of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.atherosclerosis.2020.02.002>.

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